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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF *ARTOCARPUS LACUCHA* LEAVES

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ABSTRACT

Phytochemical screening test shows the presence of Saponin, Glycoside, Flavonoids, Tannin, Triterpenoids and Phenolic compound. Free radicals can cause some major confounded illnesses like Diabetes mellitus, coronary disease, Cancer. The current examination was intended to assess the cancer antioxidant properties of ethanol extract of *Artocarpus lacucha* leaves. Free radical scavenging activity was assessed utilizing 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The IC50 of the ethanol *Artocarpus lacucha* leaves extract was 49.89µg/ml and that of ascorbic acid was 6.14µg/ml. Total Flavonoid content of the *Artocarpus lacucha* leaves extract was figure evoking the equation and discovered to be 811.9mg QE/gm dry extract. Total phenolic content of the *Artocarpus lacucha* leaves extract was determined utilizing the equation and discovered to be 164.4mg GAE/gm dry extract. Total tannin content of the *Artocarpus lacucha* leaves extracts was determined utilizing the equation and discovered to be 400.87mg GAE/gm dry extract. The examination uncovers that the *Artocarpus lacucha* leaves utilization would apply a few valuable impacts by prudence of their significant antioxidant activity.

KEYWORDS: *Artocarpus lacucha*, Antioxidant Activity, Free Radicals, Flavonoid, Phenolic, Tannin.

INTRODUCTION

Oxidative pressure can be characterized as an awkwardness between the fundamental sign of responsive oxygen species and a natural framework's capacity to promptly detoxify the receptive intermediates or to fix the subsequent harm. In people, oxidative pressure is believed to be answerable for improvement of cancer.[1] Parkinson's illness, Alzheimer's disease,[2][3] atherosclerosis, heart failure,[4] myocardial infarction,[5][6] delicate X syndrome.[7] Sickle Cell Disease,[8] lichen planus,[9] vitiligo,[10] autism,[11] infection,[12] and ongoing weakness syndrome.[13] Antioxidants terminate the chain reactions of oxidation by eliminating free extreme intermediates, and hinder other oxidation responses. Antioxidants inhibit oxidation by being oxidized it selves, so they are frequently decreasing specialists, for example, thiols, ascorbic corrosive (vitamin C), or polyphenols.[14] Antioxidants are generally utilized in dietary enhancements just as it has been demonstrated that they are viable for the anticipation of infections like malignancy, coronary illness and height sickness.[15] For the most part bioactive plant metabolites are liable for the remedial properties of the therapeutic plants.[16] Phenolic compounds mostly flavonoids and phenolic acids, have a few organic properties including antioxidant, antibacterial, anticancer activities.[17] The antioxidant limit of plant phenolics relies upon their focus,[18] number and position of hydroxyl gathering. These types of compounds are answerable for balance of destructive free extremists. Because of overproduction of free extremists and absence of enemies of oxidants a condition known as oxidative pressure is created. Freeradicalinducedoxidative damage has for quite some time been believed to be the main source of numerous ongoing and degenerative sicknesses like diabetes, stroke, malignancy, arteriosclerosis, and cardiovascular diseases.[19] These risky conditions can be defeated through a few plant optional metabolites including alkaloids, flavonoids, lignin's, phenolic compounds and terpenoids.[20] Likewise, phenolic compounds are frequently considered to assume a significant part in protection from many plant microbes.[21]

MATERIALS AND METHOD

Plant Materials

The plant *Artocarpus lacucha* leaves was collected from local market, Dhaka, Bangladesh.

PREPARATION OF THE CRUDE EXTRACT

Cold Extraction (Ethanol Extraction)

The collected plant parts (leaves) were separated from undesirable materials or plants or plant parts. They were dried in the sun for one week after cutting into small pieces. The plant parts were ground into coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark

and dry place until analysiscommenced.

About 180 gm of powdered sample was taken in a clean, flat-bottomed glass container and soaked in 1000 ml of 90% Ethanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by apiece of clean, white cotton material. Then it was filtered through Whatman filter paper. The filtrate was kept in an open space to evaporate the solvent thus crude extract was obtained.

Phytochemical Screening[22-24]

Phytochemical studied of ethanol extract of plant material extract was carried out for preliminary chemical investigation for the direction of practical pharmacognosy text book.

Screening for the Antioxidant Activity

Antioxidant activity of the extract was determined on the basis of their scavenging potential of the stable DPPH free radical in quantitative assay.

DPPH Free Radical in Quantitative Assay[25-27]

Stock solution of the plant extract was prepared in ethanol (10mg/ml) from which a serial dilution was carried out. At first 6 volumetric flasks are taken to make 6 different types of concentration 1, 5, 10, 50, 100 and 500 µg/ml. Test tubes and volumetric flasks are rapped with foil paper. In 6 volumetric flasks serial dilution of extract is done and marked themrespectively.

1ml of sample from each concentration and 3ml of 0.004% DPPH solution is taken with the help of pipette in 6 test tubes respectively. Then solution is kept in dark place for 30 minutes with raping each test tube with foil paper. In another test tube 3ml 0.004% DPPH &1ml ethanol is taken to prepare blank solution. Then absorbance is taken by UV Spectroscopy. The percent of inhibition is calculated by using followingformula__

$$\% \text{ inhibition} = \frac{\text{Blank absorbance} - \text{Solution absorbance}}{\text{Blank absorbance}} \times 100$$

Total Flavonoids Content

Total flavonoid content was estimated using aluminum chloride colorimetric assay.[28] In 1mL of the extract solution (1mg/mL), 0.2mL aluminum chloride (1% *w*/*v*), 0.2 mL potassium acetate (1 M) and 5.4 mL distilled water were added and mixed well. Then absorbance was measured at 415nm against blank solution. For this assay, quercetin (0.1–0.5mg/mL) was used to prepare standard calibration curve and total flavonoid content of the extract was expressed in terms of mg quercetin equivalent QE/gm of dried extract.

Total Phenolic Content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu's method.[29] Ethanol solution of the extract (1mg/mL) was mixed with 5mL of 10%(*v*/v) Folin-Ciocalteure agent. Then 4mL sodium carbonate (75g/L) was added to the mixture. It was kept at 40 °C for 30 min. Absorbance of the reaction mixture was measured at 765 nm. Different concentrations (0.1–0.5 mg/ mL) of gallic acid were used to prepare the standard calibration curve from where total phenol content was determined and expressed as mg gallic acid equivalent GAE/gm of dry extract.

Total Tannin Content

The tannin content of the extracts was determined by Folin Ciocalteu method.[30] In 0.1mL of the extract solution, 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent, 1 mL of 35% (*w*/*v*) Na_2CO_3 solution were added and diluted 10mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20-100µg/mL) were prepared for standard calibration curve. Absorbance for test and standard solutions were measured against suitable blank at 725 nm. The tannin content was expressed in terms of mg of GAE/gm of the dry extract.

RESULT AND DISCUSSION

Phytochemical Screening

Results of the phytochemical screening of the Ethanol Extract of *Artocarpus lacucha* leaves

Table: 1. Results of Phytochemical Screening.

Chemical Groups	Ethanol Extract of *Artocarpus lacucha* leaves
Saponin	+
Glycoside	+
Flavonoids	+
Tannin	+
Alkaloids	-
Triterpenoids	+
Phenolic compound	+

Note: (**+**) = Indicates the presence and (**−**) = Indicates the absence of the tested group.

Result of Anti-oxidants Test

DPPH Scavenging Assay

Table 2: % Inhibition of Ascorbic acid and *Artocarpus lacucha* leaves.

Conc. (µg/ml)	Absorbance (nm)			% of Inhibition	
	Blank	Ascorbic Acid	*Artocarpus lacucha* leaves	Ascorbic Acid	*Artocarpus lacucha* leaves
1	0.712	0.612	0.640	14.50	10.11
5		0.438	0.555	38.50	22.05
10		0.163	0.505	77.10	29.07
50		0.075	0.287	89.46	59.69
100		0.073	0.269	89.74	62.22
500		0.062	0.239	91.29	66.43

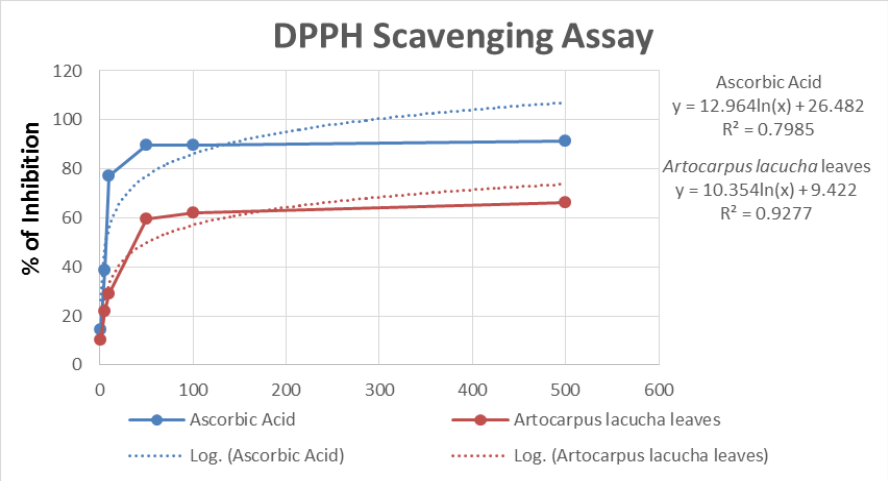

Fig. 1: Anti-oxidant activity of ascorbic acid and *Artocarpus lacucha* leaves.

Table 3: IC_{50} values of the extracts of Ascorbic Acid and *Artocarpus lacucha* leaves.

Test Samples	Regression line	R^2	IC_{50} (µg/ml)
Ascorbic Acid	y = 12.96ln(x) + 26.48	R^2 = 0.798	6.14
Artocarpus lacucha leaves	y = 10.354ln(x) + 9.422	R^2 = 0.927	49.89

Discussion

The antioxidant activity of the ethanol extract *Artocarpus lacucha* leaveswas evaluated using DPPH free radical scavenging activity method. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts.[31-32] Ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine.[33-34] The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties.[35] The ethanol extract of *Artocarpus lacucha* leaveshas significant anti-oxidant activity. The $IC_{50}$of the *Artocarpus lacucha* leavesis 49.89µg/ml, whereas $IC_{50}$of Ascorbic Acid is 6.14 µg/ml.

Total Flavonoids Content.

Concentration (mg/ml)	Absorbance (nm)
0.1	0.138
0.2	0.146
0.3	0.14
0.4	0.178
0.5	0.248

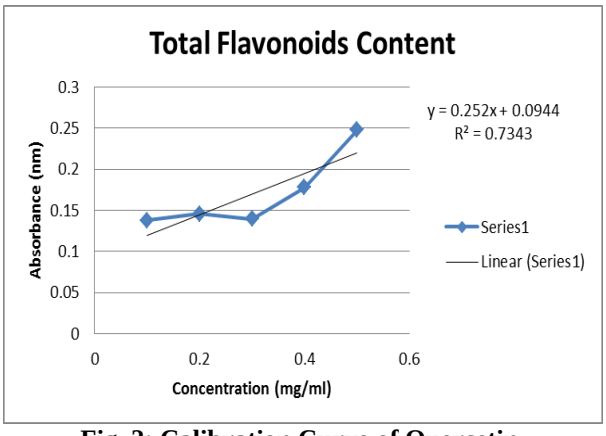

Fig. 2: Calibration Curve of Quercetin.

Discussion

Also, the absorbance values obtained in the test using different concentrations of quercetin were plotted against respective concentrations. A standard calibration curve was obtained with the equation y = 0.252x + 0.0944 (R^2 = 0.7343). Total Flavonoid content of the *Artocarpus lacucha* leaves extract was calculate educing the equation and found to be 811.9mg QE/gm dry extract.

Total Phenolic Content.

Concentration (mg/ml)	Absorbance (nm)
0.1	0.366
0.2	0.947
0.3	1.616
0.4	1.964
0.5	2.504

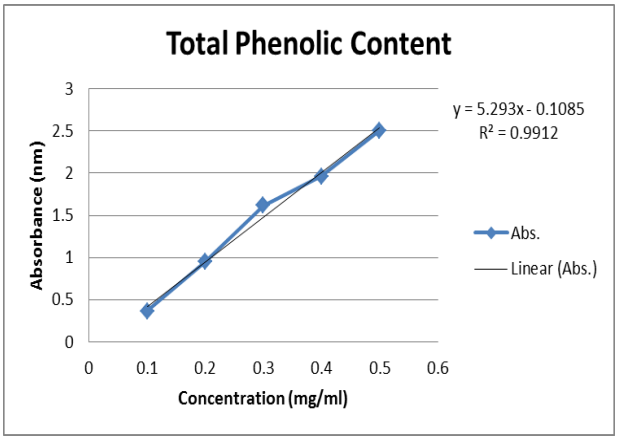

Fig. 3: Calibration Curve of Gallic Acid.

Discussion

In case of total phenolic content, the absorbance values obtained in the test using different concentrations of gallic acid were plotted against respective concentrations. A standard calibration curve was obtained with the equation y = 5.293x - 0.1085 (R^2 = 0.9912). Total phenolic content of the *Artocarpus lacucha* leaves extract was calculated using the equation and found to be 164.4mg GAE/gm dry extract.

Total Tannin Content.

Concentration (mg/ml)	Absorbance (nm)
0.1	0.918
0.2	1.374
0.3	1.681
0.4	1.914
0.5	2.251

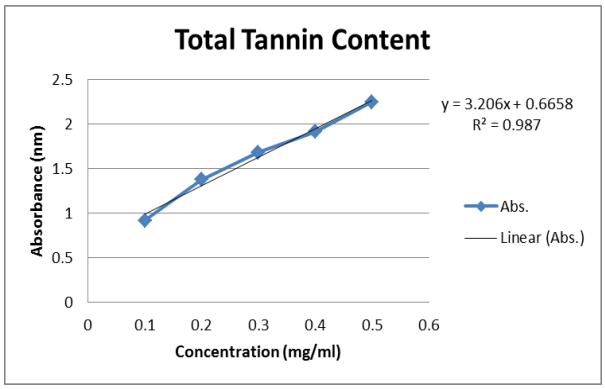

Fig. 4: Calibration Curve of Gallic Acid.

DISCUSSION

On the other hand, the absorbance values obtained in the total tannin content test using different concentrations of gallic acid were plotted against respective concentrations. A standard calibration curve was obtained with the equation y = 3.206x + 0.6658 (R^2 = 0.987). Total tannin content of the *Artocarpus lacucha* leaves extracts was calculated using the equation and found to be 400.87mg GAE/gm dry extract.

CONCLUSION

The present study revealed that extracts of the *Artocarpus lacucha* leaves can be used as a source of antioxidant. At last, we can say that further study is needed to do *in-vivo* antioxidant activity and find out the causative metabolites of *Artocarpus lacucha* leaves and possible mechanism.

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